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SALT APPETITE DURING ACUTE SODIUM  
DEFICIENCY IN THE GERBIL

by

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Bachelor of Arts  
Villanova University

A Thesis Submitted in Partial Fulfillment of  
The Requirements for the Degree of  
Master of Arts  
in the  
Department of Psychology  
of the  
Graduate School  
University of Richmond  
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### Abstract

In the present study, two experiments were conducted to examine the gerbil's response to acute sodium deficiency. Adult male gerbils were either exposed to a 1% b.w. subcutaneous injection of 1.5% formalin or a vehicle control injection. Within each injection level, half the animals were further assigned to either an isotonic saline or a water vehicle. Immediately following the injection, each S had access to isotonic saline and water as their drinking fluid. The formalin dissolved in water group displayed a significant increase in saline consumption, but at the same time decreased their water intake, in comparison to the water vehicle injection group. However, the formalin dissolved in isotonic saline group and the isotonic saline injection group did not differ in saline intake, but the formalin group drank significantly more water. Therefore, it was concluded that the gerbil's response to formalin is dependent upon the type of vehicle in which formalin is dissolved. It was postulated that these differences in intake between the two formalin groups may be attributed to differences in physiological changes produced by formalin, or to differences in precedence of volume or osmoregulation. In the second experiment, adult male gerbils were exposed to a 1% b.w. subcutaneous injection of 1.5% formalin or to a vehicle injection.

Within each injection level, the animals were further divided so that equal groups had access to water and either .45%, .9%, 1.8%, or 3.6% saline solution or to water alone. The formalin group, who had access to water alone, significantly increased water consumption 12 and 24 hours after injection. Furthermore, gerbils significantly increased their intake of water 6 and 12 hours after formalin injection, when faced with a two-bottle choice situation, in comparison to a vehicle group. However, contrary to expectation, no increases in consumption of saline for the formalin group was evident. Due to these findings, no definitive conclusion can be made concerning the gerbil's ability to regulate sodium intake when various concentrations of saline solution and water are provided. It was postulated that the gerbil's drinking behavior subserves volume regulation over osmoregulation. These findings were explained in terms of the inability to take renal defense mechanisms into account and the sodium reservoir hypothesis.

## Salt Appetite During Acute Sodium Deficiency in the Gerbil

The maintenance of body fluid homeostasis requires an adequate intake of both water and sodium. The behavioral and physiological mechanisms underlying thirst have been investigated extensively, and it appears that the sensation of thirst is dependent upon volume deficits and/or concentration increases occurring in the cellular and/or extracellular fluid compartments (Blass, 1974). On the other hand, relatively little is known about the physiological process which stimulates the appetite for sodium and its concomitant behavioral responses. In this regard, the physiological changes most frequently cited are hypovolemia (volume deficit), hyponatremia (concentration deficit), and elevated circulating levels of aldosterone (Blass, 1974).

The exact nature of the role played by these mechanisms have not been identified, since each may elicit a sodium appetite but none of them are necessary for producing sodium ingestion. Thus, hypovolemia produced by polyethylene glycol (PG) injection elicits sodium appetite in rats but only after a considerable delay (Stricker & Wolf, 1966; Stricker & Jalowiec, 1970). Further evidence confirming that hypovolemia can potentiate a sodium

to this redistribution of body fluids formalin potentiates a sodium appetite in the rat (Stricker & Wolf, 1966; Stricker, 1966; Wolf & Steinbaum, 1965; Jalowiec & Stricker, 1970). These observed increases in drinking are not due to a nonspecific thirst since, as demonstrated by Handal, formalin stimulates an appetite specific for sodium salts (1965b), and is dose-related (1965a). Further confirmatory evidence is provided by Jalowiec and Stricker (1970). These authors found that rats could restore body fluid balance, when given access to water and either hypotonic, isotonic, or hypertonic saline solution to drink. In all cases, the total fluid consumed was equivalent. However, intakes of water and saline varied depending on the concentration of the saline solution provided, with the higher concentrations producing smaller intakes.

In recent years, research delineating the physiological and behavioral mechanisms of sodium appetite in rats has grown, but substantially little attention has been directed to a comparative analysis using arid dwelling animals such as the gerbil. It seems reasonable to speculate that these animals should differ from the rat in their responses to manipulation of electrolyte balance. Cullen and Scarborough (1970) demonstrated these differences

are present, when they found that adrenalectomized gerbils were unable to regulate chronic sodium deficiency. These authors found that gerbils failed to exhibit a salt appetite, and died unless given cortical hormone therapy. These findings are in marked contrast to the rat which can survive by regulating intakes of sodium chloride following adrenalectomy (Richter, 1936). This suggests that the gerbil is a highly adrenal-dependent animal, which may account for its having an adrenal-to-body-weight ratio three times larger than the rat (Cullen, Pare, & Mooney, 1971).

There exists a paucity of research dealing with the gerbil's response to various dipsogenic challenges. Recent research has demonstrated that the gerbil responds to an extracellular stimulus of thirst in a manner similar to the rat (Almli & Weiss, 1975; Hauenstein, 1978). Furthermore, Hauenstein (1978) has found that the gerbil drinks comparably more saline than water in response to PG-treatment. However, no information was provided on the regulatory behaviors of the gerbil, since only one concentration of saline was provided, and the solutions were not present simultaneously. Little evidence concerning the gerbil's response to acute sodium deficiency exists. One exception is the work

of Cullen (1972), who performed three experiments to examine the gerbil's response to subcutaneous injections of either .025, .25, or 2.5 ml of 1.5% formalin or a vehicle control. These experiments differed on the basis of diet and drinking fluids available. These differences were: 1) sodium-deficient diet with isotonic saline and water available, 2) Purina chow for food with saline alone, and 3) Purina chow with saline and water. Cullen demonstrated that gerbils drank comparable amounts of saline in all three experiments, when given any of the injections. The author found that in comparison to the rat, the gerbil's intake of saline was similar, but its total consumption was greater. Cullen discounts the results as an effect of a formalin-induced nonspecific thirst. If the polydipsia were due to such an effect, then the total fluid ingested in each experiment should have been equivalent. However, when only saline was available consumption did not increase, and therefore the amount of liquids consumed was less.

These findings suggests that the gerbil responds to sodium deficiency in a manner comparable to the rat. However, since the response was not dose-related and the vehicle control animals also increased intake of isotonic saline, it is difficult

to assert conclusively that formalin produces a sodium appetite and not a nonspecific thirst. Methodological problems such as using a standard injection, without taking body weights into account, produces varying needs of sodium in the animals and introduces an unnecessary source of variability. Furthermore, Cullen's design does not tell us if the gerbil can regulate sodium intake, since only one concentration of saline solution was provided. Finally, no information is given regarding the gerbil's response when water is the only drinking fluid available.

In the present study, two experiments were conducted to clarify the above findings, and elucidate that the gerbil's response to sodium deficiency is characteristic of a sodium appetite and not due to a nonspecific thirst. The first experiment determined whether formalin in an isotonic saline or water vehicle should be utilized. The second experiment explored the relationship between a specific sodium need and the intakes of various concentrations of saline solution and water, and the intake of water when it was the only available fluid, during acute sodium deficiency.

### Experiment 1

#### Method

Animals. Twenty adult male gerbils obtained from Tumblebrook Farms (Massachusetts) were housed in individual cages, and were provided with constant illumination in a temperature controlled room (23-26°). The Ss had ad lib access to Purina chow and water in an inverted graduated tube with metal drinking nozzles attached to the front of the cages. A period of approximately 2 weeks was provided for pretreatment maintenance to insure stable drinking.

Procedure. For at least three consecutive days preceding treatment, measurements of body weight and fluid intake was recorded every 24 hours. At this time, body weights did not fluctuate more than 4 grams and fluid intakes remained relatively stable ( $\pm 3\text{ml}$ ). Following the establishment of the above baseline conditions, the animals were randomly assigned to either the formalin or control injection group. Within each of these groups, the animals were further assigned to either an isotonic saline or water vehicle condition. The Ss received one of the following 1% b.w. subcutaneous injection: 1) 1.5% formalin (0.6% formaldehyde adjusted to pH 7.4 with NaOH) in an isotonic saline vehicle, 2) 1.5% formalin in a water vehicle, 3) isotonic saline vehicle, or 4) water vehicle. The Ss were lightly etherized for approximately 20 seconds prior to injection time.



Immediately following the injection, each Ss had access to water and isotonic saline solution (0.9%). Water and saline intake measurements were recorded at 2, 4, 6, 12, and 24 hour periods on the first day, and then at 12 and 24 hour periods for the following 5 days.

Results. Unfortunately, there was one loss in each group due to death or unstable baseline drinking. Fluid intake measures were converted to percent body weight, and combined to 24 hour points.

Saline Intake. Mean intakes of isotonic saline are shown in Table 1, and illustrated in Figure 1. A 2x2x6 ANOVA with repeated measures on the third factor was used to analyze the data, and the summary of results is depicted in Table 2. As was predicted, there was a significant interaction associated with treatment and type of vehicle. This finding permitted the investigation of simple effects to determine whether there was a significant difference between treatments at each vehicle level and across each treatment. The summary of results for the analysis of simple effects for differences between treatments at each vehicle level is presented in Table 3a. This interaction is depicted in Figure 2. The analysis of simple effects indicated a significant treatment effect. As was hypothesized, formalin dissolved in

Table 1

Mean Intake of Saline Expressed as % B.W.  
1,2,3,4,5, & 6 Days After Injection

		<u>Time After Injection</u>					
		1	2	3	4	5	6
<u>Injection</u>	F + H <sub>2</sub> O	9.33	8.43	11.55	8.40	9.45	7.68
	H <sub>2</sub> O	3.00	1.28	1.33	.48	2.58	2.43
	F + NaCl	4.00	1.55	3.40	1.58	1.88	2.38
	NaCl	6.20	2.73	4.73	1.15	5.63	4.70

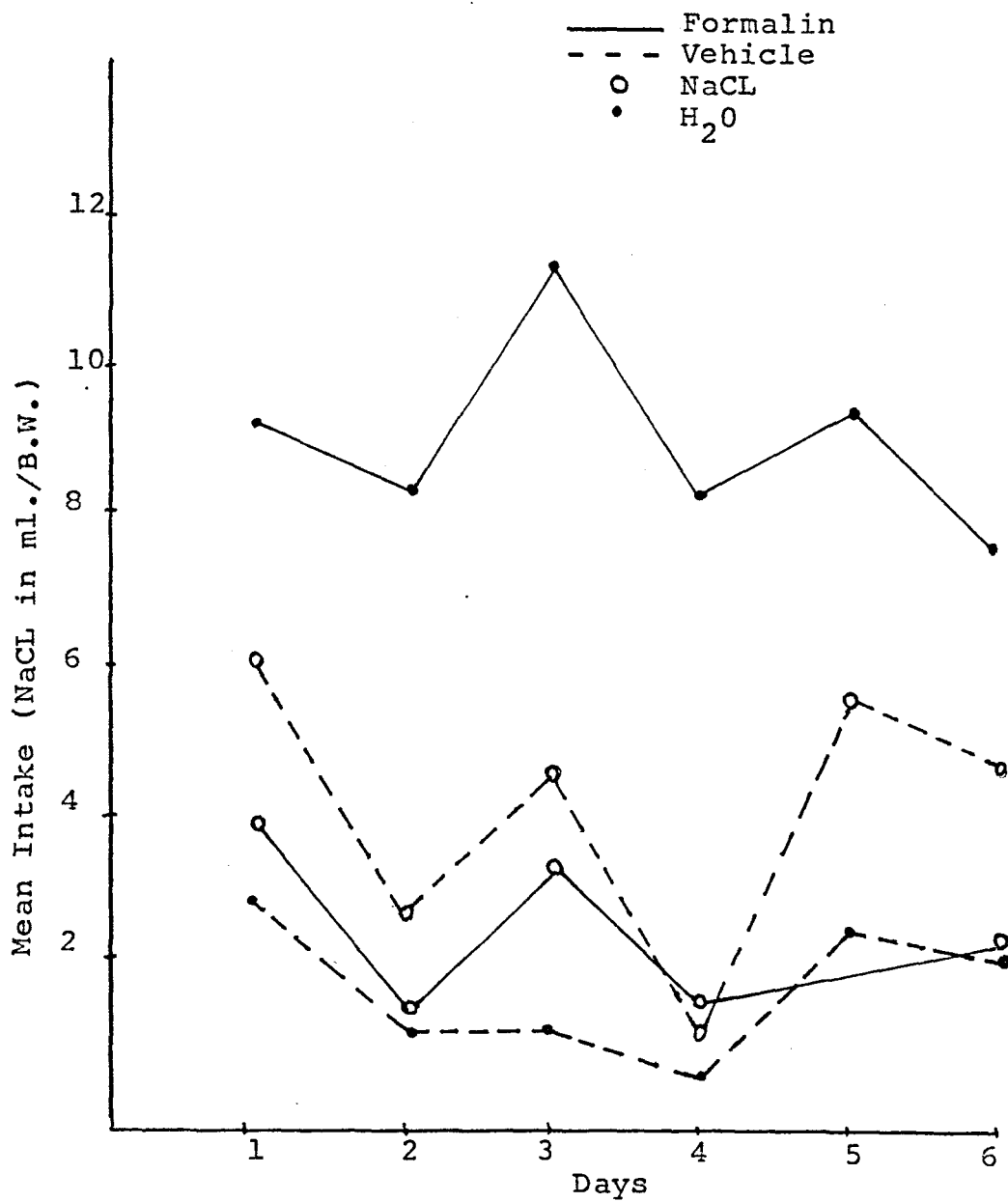


Figure 1

Mean Intakes (NaCL in ml./B.W.) at 1,2,  
3,4,5, & 6 Days After Injection

Table 2

Analysis of Variance: Saline Intake  
Expressed as % B.W.

Source	df	MS	F
<u>Between Ss</u>	15		
Treatment	1	185.92	2.66
Vehicle	1	112.66	1.61
Treatment X Vehicle	1	487.81	6.98*
Error(b)	12	69.85	
<u>Within Ss</u>	80		
Trials	5	17.77	2.83*
Trials X Treatment	5	6.81	1.08
Trials X Vehicle	5	2.26	.36
Trials X Vehicle X Treatment	5	2.82	.45
Error(w)	60	6.28	

\*p < .05

Table 3aAnalysis of Variance: Saline  
Intake Simple Effects

Source	df	MS	F
Treatment at H <sub>2</sub> O Vehicle	1	3823.13	54.77**
Treatment at NaCl Vehicle	1	214.25	3.07
Error	12	69.89	

Table 3bAnalysis of Variance: Saline  
Intake Simple Effects

Source	df	MS	F
Treatments	1	3208.01	45.9**
Vehicles	1	394.81	5.65*
Error	12	69.89	

\* $p < .05$ \*\* $p < .01$

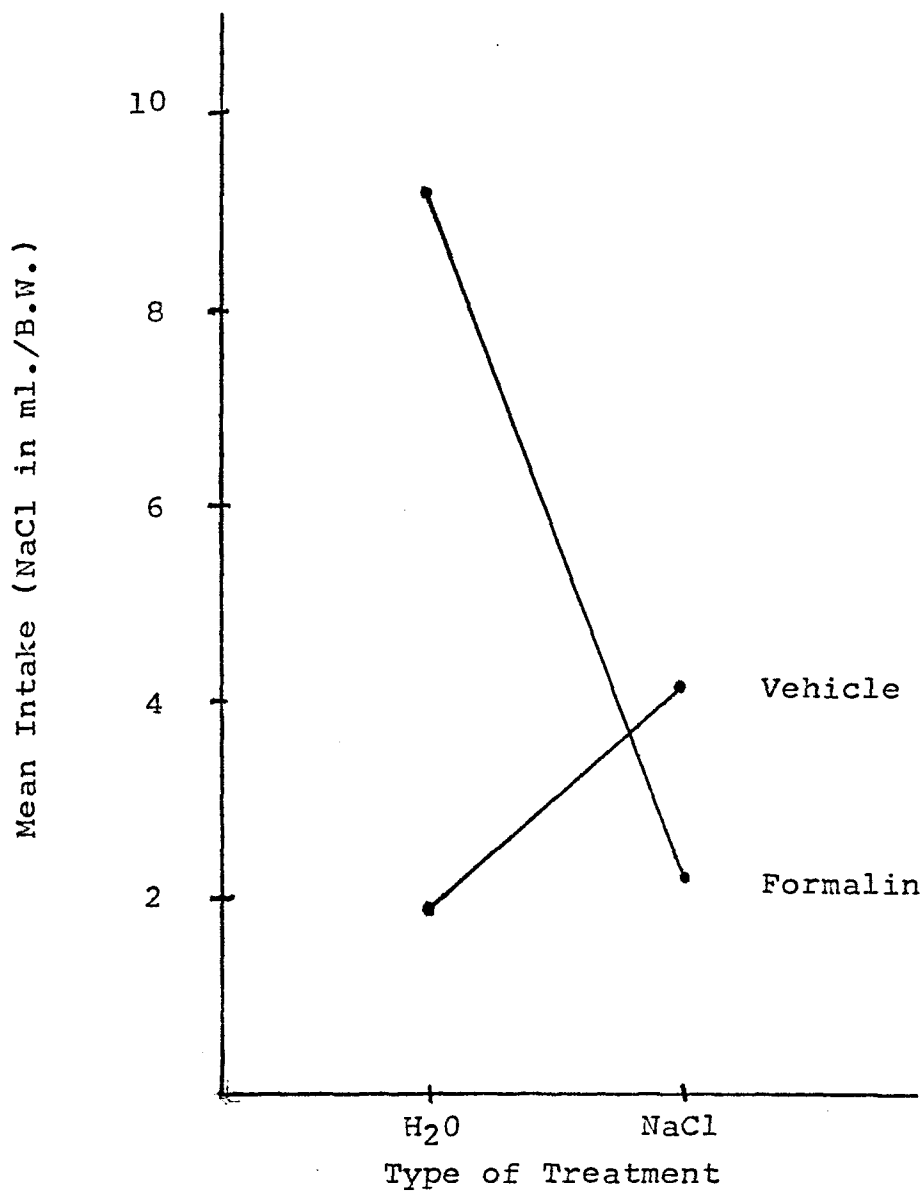


Figure 2  
Mean Intakes (NaCl in ml./B.W.) for  
each Treatment Group as a Function  
of Type of Vehicle

water produced greater intakes of saline, then the vehicle injection. However, formalin dissolved in isotonic saline and the isotonic saline vehicle injection did not produce significant differences in intake. The summary of results for the analysis of simple effects across treatment levels is presented in Table 3b. This interaction is illustrated in Figure 3. Examination of the analysis of simple effects disclosed a significant difference between the two formalin groups. Significantly larger intakes of saline were attributed to the formalin dissolved in water group, then to the formalin dissolved in isotonic saline group. Furthermore, significant differences in intake were found between the two vehicle groups. Here, isotonic saline vehicle injections produced greater saline intakes in comparison to the water vehicle injection. Finally, a Newman-Keuls test revealed that the main effects of trials was due to the significantly larger intakes on the first day than the fourth day. The results of this Newman-Keuls test is presented in Table 4.

Water Intake. Mean intakes of water are shown in Table 5, and illustrated in Figure 4. A 2x2x6 ANOVA with repeated measures on the third factor was used to analyze the data. The summary of results is depicted in Table 6. Again, there was a

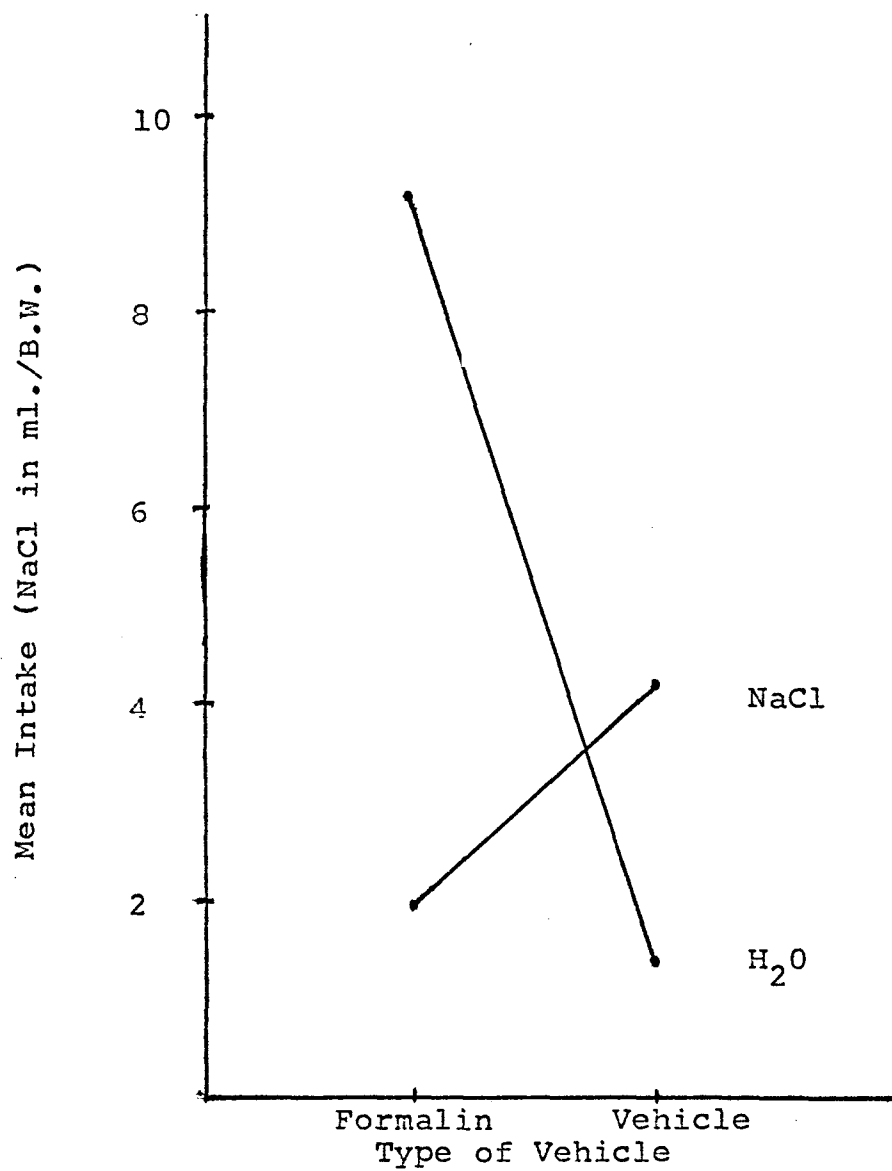


Figure 3

Mean Intakes (NaCl in ml./B.W.) for each  
Vehicle Group as a Function  
of the Type of Treatment



Table 4

Newman-Keuls: Saline Intake--  
Trials Main Effect

<u>Trial</u>					
4	2	6	5	3	1
2.9	3.49	4.29	4.88	5.25	5.63
	.59	1.39	1.98	2.35	2.73*
		.8	1.39	1.76	2.14
			.59	.96	1.34
				.37	.75
					.38

\* $p < .05$

Table 5

Mean Intakes of Water Expressed as % B.W.  
1,2,3,4,5, & 6 Days After Injection

		<u>Time After Injection</u>					
		1	2	3	4	5	6
<u>Injection</u>	F + H <sub>2</sub> O	6.26	4.45	4.73	6.63	7.37	8.75
	H <sub>2</sub> O	11.93	11.86	14.75	14.08	15.94	15.98
	F + NaCl	11.35	16.5	15.74	15.09	14.63	12.41
	NaCl	7.74	10.29	8.04	9.6	9.1	10.86

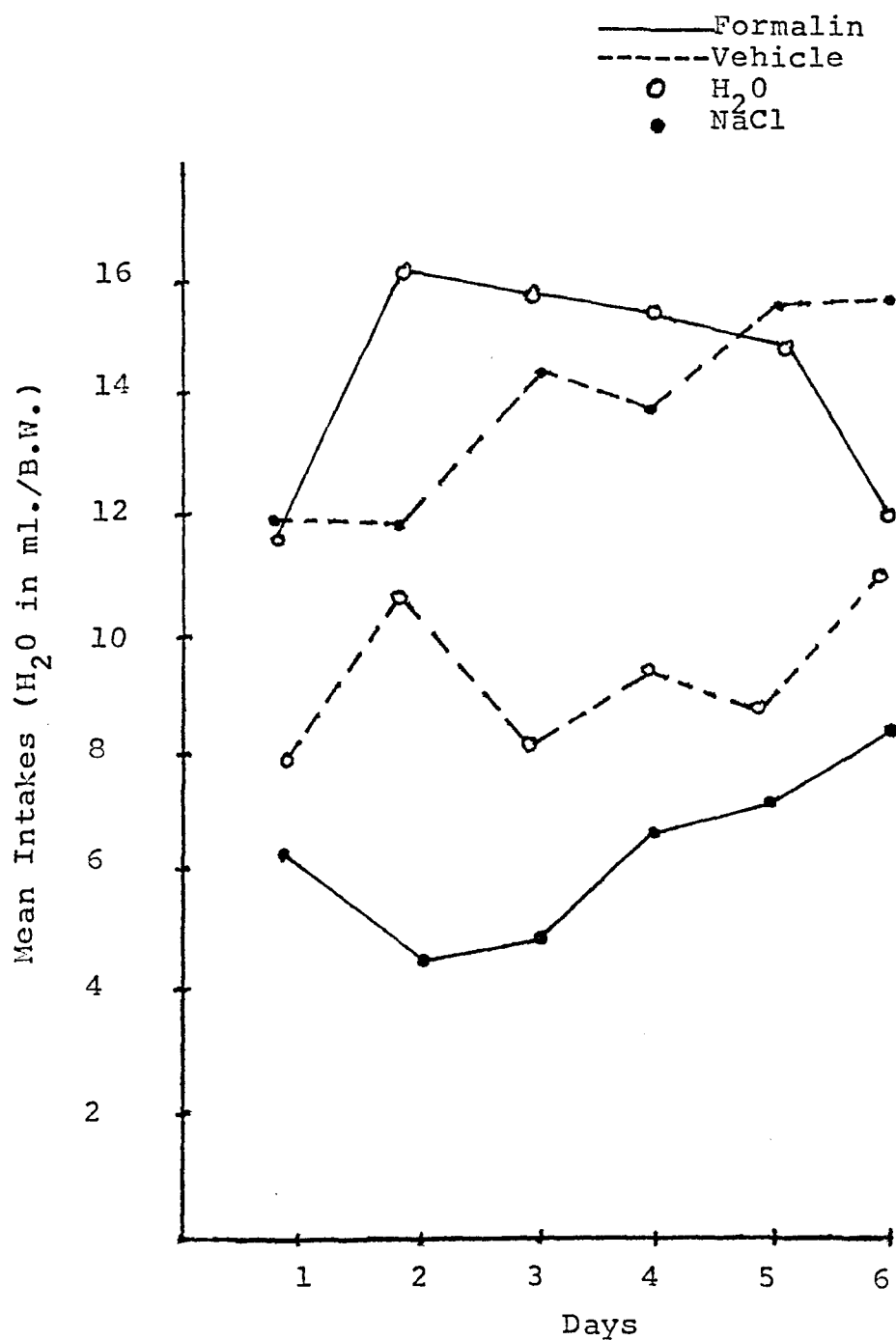


Figure 4

Mean Intakes (H<sub>2</sub>O in ml.B.W.) at 1,2,3,  
4,5, & 6<sup>2</sup>Days After Injection

Table 6

Analysis of Variance: Water Intake  
Expressed as % B.W.

Source	df	MS	F
<u>Between Ss</u>	15		
Treatment	1	44.01	.29
Vehicle	1	57.91	.38
Treatment X Vehicle	1	973.97	6.33*
Error <sub>(b)</sub>	12	153.76	
<u>Within Ss</u>	80		
Trials	5	14.72	2.27
Trials X Treatment	5	2.46	.38
Trials X Vehicle	5	17.91	2.76*
Trials X Vehicle X Treatment	5	11.0	1.69
Error <sub>(w)</sub>	60	6.49	

\*p < .05

significant interaction associated with treatment and the type of vehicle. This interaction allowed the investigation of simple effects to determine whether there was a significant difference between the two treatments at each vehicle level and across treatments. The analysis of simple effects for differences between treatments at each vehicle level is presented in Table 7. This interaction is depicted in Figure 5. The analysis of simple effects indicated a significant difference between treatments at each vehicle level. In particular, the water vehicle injection produced greater intakes, then the formalin dissolved in a water vehicle; whereas the formalin dissolved in isotonic saline produced greater intakes then the isotonic saline vehicle injection. The summary of results for the analysis of simple effects across treatments is presented in Table 8. This interaction is shown in Figure 6. The analysis of simple effects revealed a significant difference between the two formalin groups and the two vehicle groups. More specifically, formalin dissolved in isotonic saline produced a significantly larger intake of water in comparison to formalin dissolved in water. Furthermore, the water vehicle produced greater intakes then the isotonic saline vehicle.

The significant interaction associated with

Table 7Analysis of Variance: Water  
Intake Simple Effects

Source	df	MS	F
Treatment at H <sub>2</sub> O vehicle	1	4296.18	27.94**
Treatment at NaCl Vehicle	1	1811.72	11.78**
Error	12	153.76	

Table 8Analysis of Variance: Water  
Intake Simple Effects

Source	df	MS	F
Treatments	1	4520.58	29.4**
Vehicles	1	1670.71	10.87**
Error	12	153.76	

\*\*p < .01

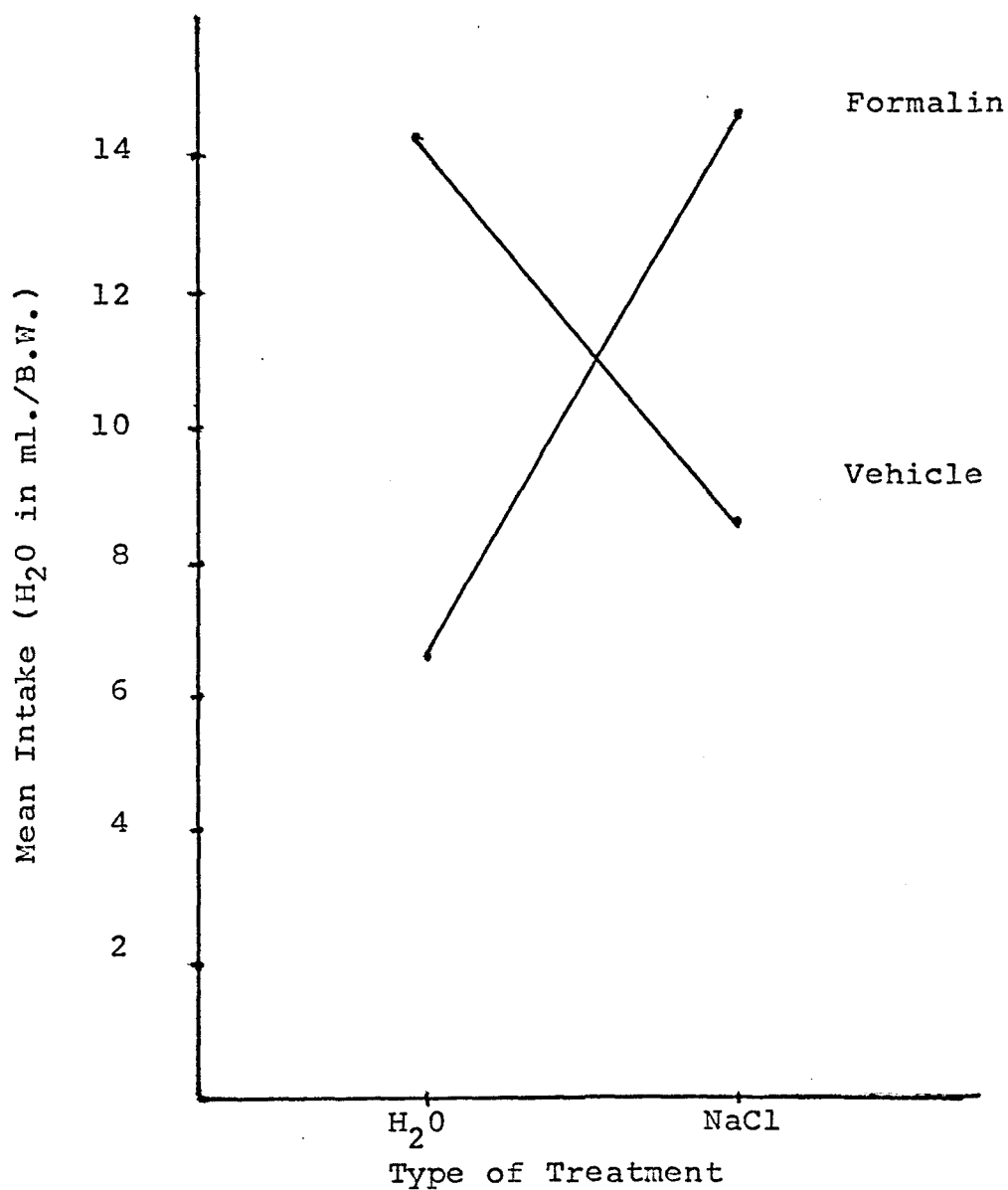


Figure 5

Mean Intakes (H<sub>2</sub>O in ml./B.W.) for  
each Treatment Group as a Function of  
the Type of Vehicle

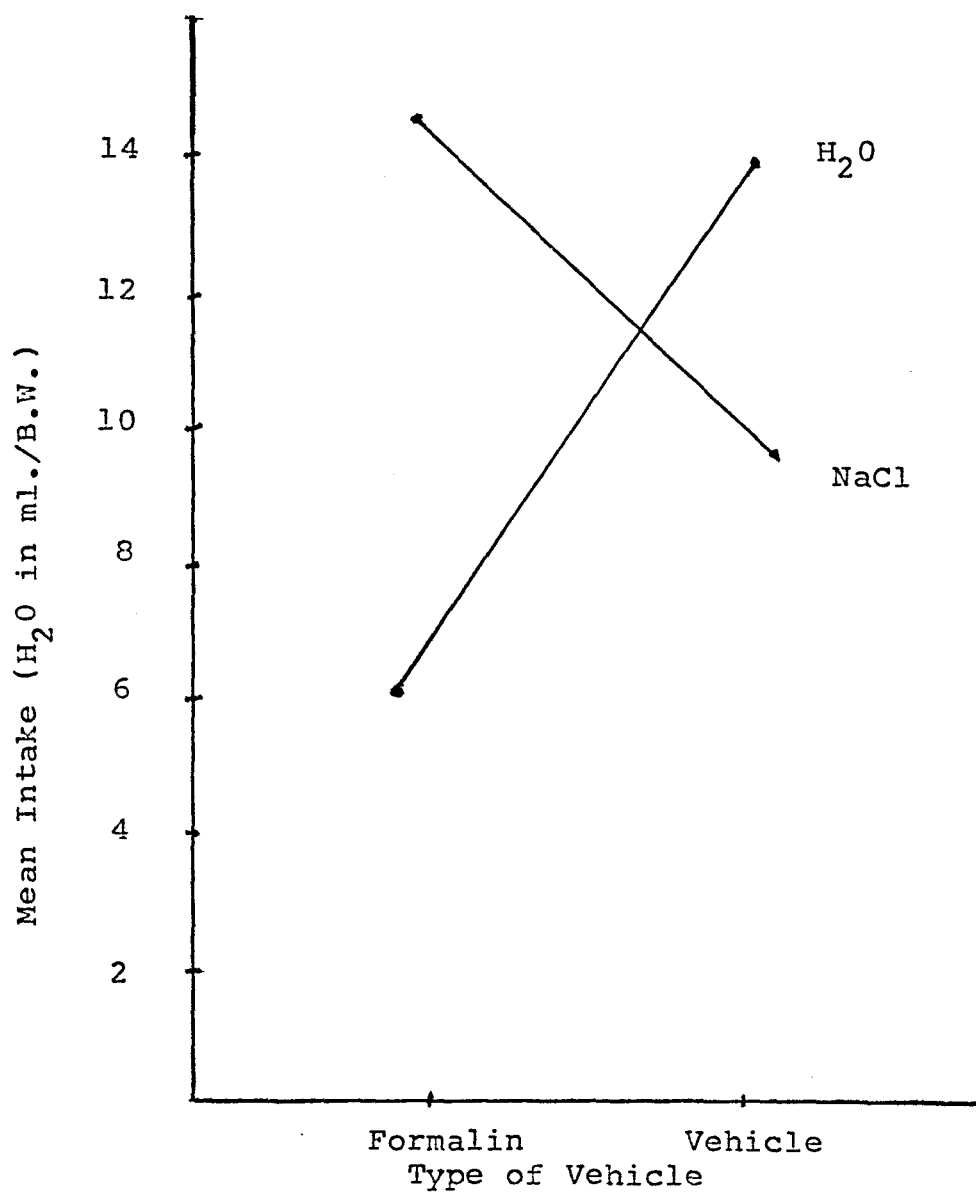


Figure 6

Mean Intakes ( $H_2O$  in ml./B.W.) for  
each Vehicle Group as a Function  
of the Type of Treatment



type of vehicle and trials allowed the investigation of simple effects to determine whether there was a difference between the treatment groups at each trial. This interaction is illustrated in Figure 7, and the summary of results is shown in Table 9. The analysis disclosed a significant trials effect for the water vehicle group, but not for the isotonic saline groups. A Newman-Keuls test was performed on the means of the water vehicle groups across the trials. The results of this test are displayed in Table 10. It disclosed a significant increase in intake between the first day and the fifth day, between the first day and the sixth day, between the third day and the fifth day, between the third day and the sixth day, and between the fifth day and the sixth day.

#### Discussion

The results of the present study demonstrate that the gerbil's drinking in response to formalin is dependent upon the type of vehicle in which formalin is dissolved. This finding is in marked contrast to the rat, who displays similar increases in intake of saline, when injected with formalin dissolved in either vehicle (Wolf & Steinbaum, 1965; Jalowiec & Stricker: 1970). As was hypothesized, formalin dissolved in water produced a significant increase in saline intake, in comparison to a comparable

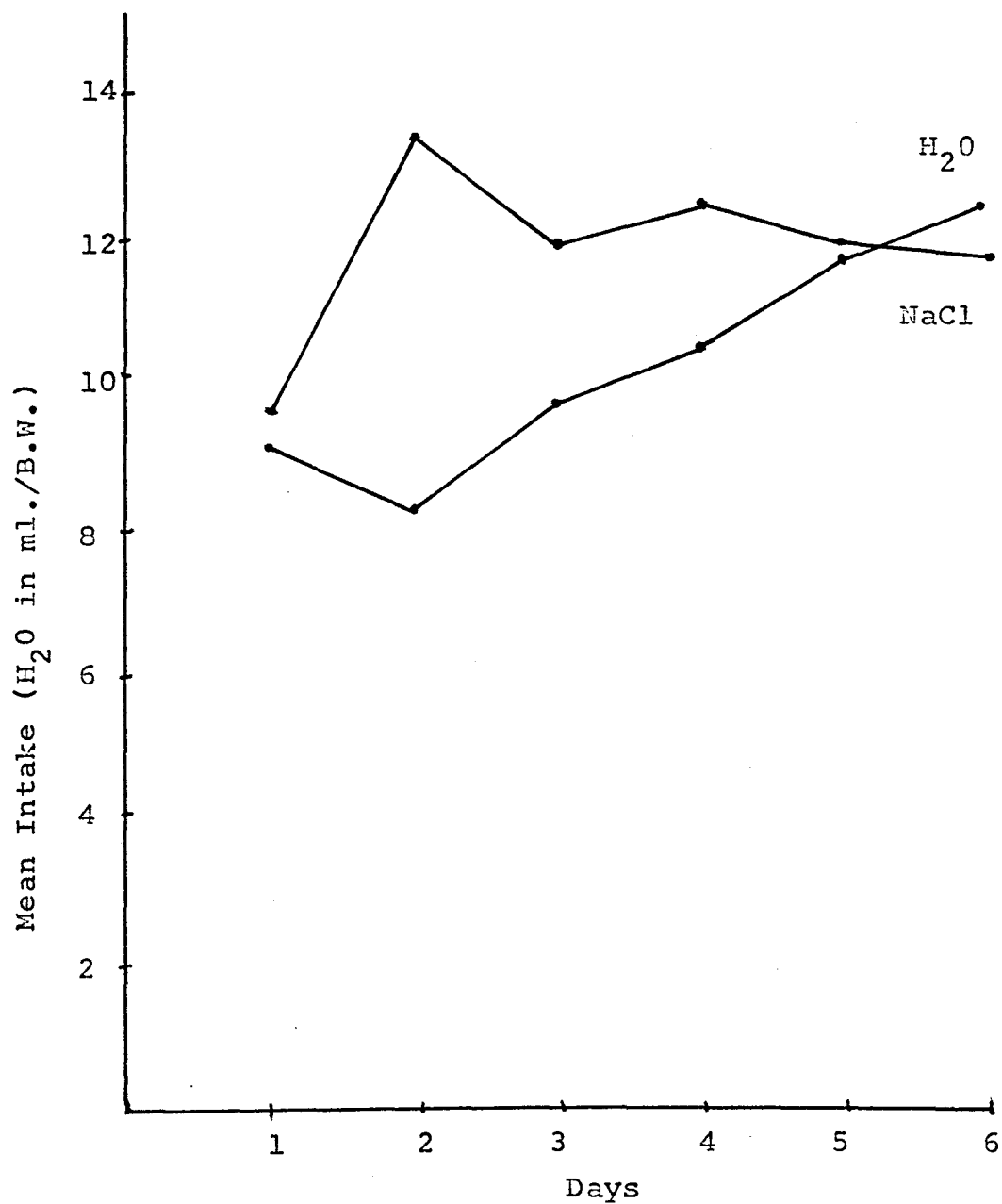


Figure 7

Mean Intake (H<sub>2</sub>O in ml./B.W.) for each  
Treatment Group as a Function  
Of Trials(Days)

Table 9

Analysis of Variance: Water In-  
take Simple Effects

Source	df	MS	F
Trials at H <sub>2</sub> O Vehicle	5	19.91	3.07*
Trials at NaCl Vehicle	5	12.73	1.96
Error	60	6.49	

Table 10

Newman-Keuls: Water Intake  
Simple Effects

<u>Trials</u>					
2	1	3	4	5	6
8.15	9.1	9.74	10.35	11.65	12.36
	.95	1.59	2.2	3.5*	4.21*
		.64	1.25	2.55*	3.26*
			.61	1.91	2.62*
				1.3	2.01
					.71

\*p < .05

control injection, with peak consumption occurring on the third day as can be seen in Figure 1. At the same time, water intakes were significantly lower in the formalin group than the vehicle group. On the other hand, the formalin dissolved in an isotonic saline and its vehicle control group did not show equivalent increases in the consumption of saline, but instead showed no increase. However, this formalin group significantly increased their intake of water in comparison to its vehicle group. These findings, concerning the effects of formalin in an isotonic saline vehicle, differ from Cullen (1972), who found that this injection produced significant increases in saline consumption.

Direct comparison between the two formalin groups reveals that formalin dissolved in water produces greater increases in saline intake in the gerbil than formalin dissolved in isotonic saline. On the other hand, formalin dissolved in isotonic saline causes the animals to increase water consumption in comparison to formalin dissolved in water. These differences in the behavioral responses suggest that maybe different physiological changes are taking place. The increases in water intake associated with formalin dissolved in saline, suggest that the gerbil is drinking to remediate only a plasma volume deficit,

or that this deficit is greater to a substantial degree. This is not a likely explanation, since Hauenstein (1978) found that gerbils drinking to remediate plasma volume deficits drank more saline than water. However, in this experiment saline intake was minimal. On the other hand, increases in saline intake associated with formalin dissolved in water, suggest that the gerbil is drinking to repair both plasma volume and concentration deficits. This implies that this injection produces both plasma volume and concentration deficiencies.

An alternate explanation is that these differences in fluid intake reflect differences in precedence of volume or osmoregulation. In particular, formalin dissolved in saline causes drinking to subserve volume regulation, whereas formalin dissolved in water--osmoregulation.

Due to the finding that only formalin dissolved in water results in increased saline consumption in comparison to its vehicle, it was decided that this injection was to be used in the second experiment.

#### Introduction

The following experiment was conducted to examine the relationship between a specific sodium need, and the intake of various concentrations of saline solutions and water, and the intake of water when it

is the only available fluid, during acute sodium deficiency in the gerbil. If the gerbil's drinking behavior is characteristic of a sodium appetite, then the gerbil should be able to regulate intakes of sodium and water so that varying amounts of saline solution are consumed depending on the concentration of saline provided. Therefore, all groups should exhibit equivalent intakes in sodium to remediate their plasma volume and concentration deficits.

## Experiment 2

### Method

Animals. Seventy adult male gerbils were obtained and housed as in Experiment 1.

Procedure. The pretreatment maintenance was the same as in Experiment 1, and the same baseline conditions were established. The animals were randomly assigned to either the formalin or control injection group. Within each of treatment levels, the Ss were further divided so that equal groups had access to water and either .45%, 0.9%, 1.8%, or 3.6% saline solution or to water alone. This results in 10 independent groups with 7 Ss in each group. The Ss received 1% b.w. subcutaneous injection of 1.5% formalin (0.6% formaldehyde in water adjusted to

pH 7.4 with NaOH) or vehicle control. Prior to injection, the Ss were lightly etherized for approximately 20 seconds. Immediately following the injection, each S had access to water and a particular concentration of saline solution or water alone as the drinking fluid depending on the group they were in. Measurement of water and saline intake were the same as in Experiment 1.

Results. Fluid intake measures were expressed as a percentage of body weight. All intake measures were combined to 24 hour points unless indicated otherwise.

Water Intake--Water Alone Groups. Mean intakes of water at 2, 4, 6, 12, and 24 hours after injection are presented in Figure 8. The means are also presented in Table 11. A 2x5 ANOVA with repeated measures on the second factor was used to analyze the experimental data, and this analysis is shown in Table 12. The analysis of variance revealed significant main effects for treatment and trials. A Newman-Keuls test was performed to determine where the differences across trials occurred. The Newman-Keuls summary table is presented in Table 13. This test disclosed a significant increase between all preceding trials and 12 hours, and between all preceding trials and 24 hours.

The mean intakes of water for 1, 2, 3, 4, 5, and 6

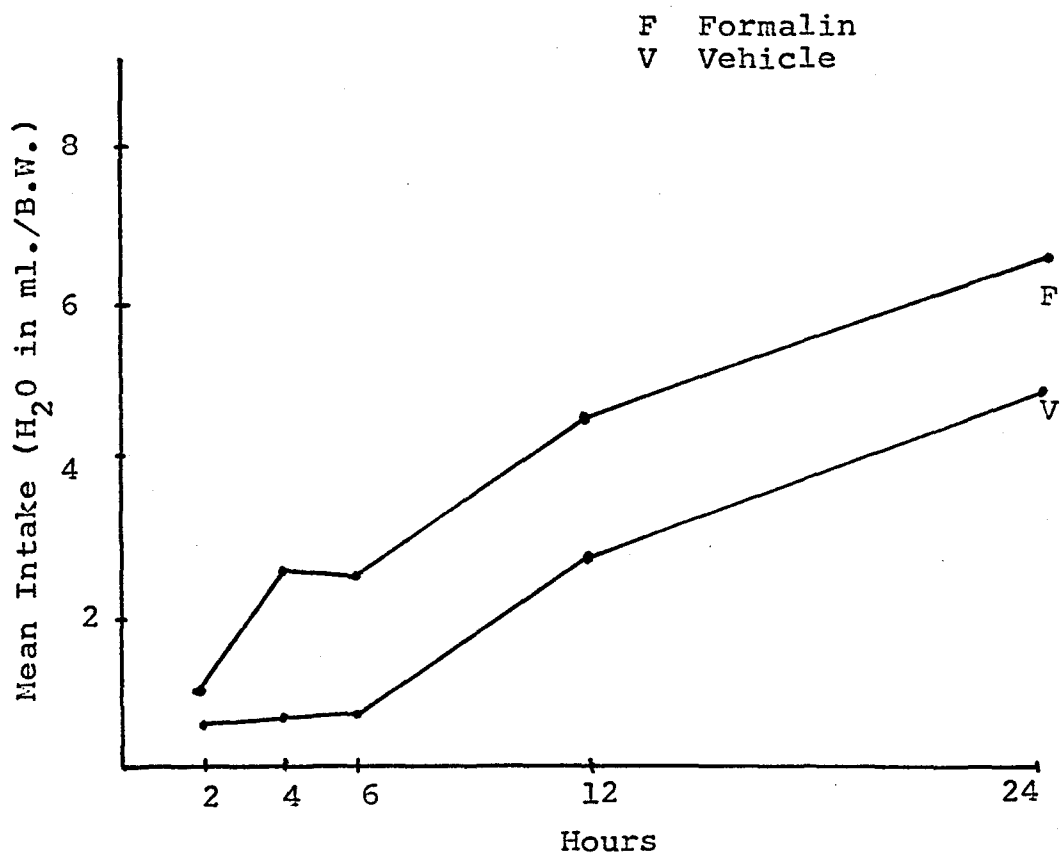


Figure 8

Mean Intakes ( $H_2O$  in ml./B.W.) for  
Each Treatment Group at 2, 4,  
6, 12, & 24 Hours After In-  
jection



Table 11

Mean Intake of Water Expressed as % B.W.  
2,4,6,12, & 24 Hours After Injection

	<u>Time After Injection</u>				
	2	4	6	12	24
Formalin	.87	2.55	2.35	4.45	6.86
Vehicle	.43	.48	.65	2.68	4.79

Table 12

Analysis of Variance: Water Intake Expressed  
as % B.W.

Source	df	MS	F
<u>Between Ss</u>	13		
Treatment	1	42.51	17.21**
Error (b)	12	2.47	
<u>Within Ss</u>	56		
Trials	4	60.48	28.8**
Trials X Treatment	4	1.56	.74
Error (w)	48	2.1	

\*p < .05

\*\*p < .01

Table 13

Newman-Keuls: Trials Main Effect

<u>Trials</u>				
1	2	3	4	5
.65	1.5	1.52	3.44	5.83
	.85	.87	2.79*	5.18*
		.02	1.94*	3.66*
			1.92*	4.31*
				2.39*

\*p &lt; .05

days after injection are presented in Table 14, and depicted in Figure 9. A 2x6 ANOVA with repeated measures on the second factor was utilized to analyze the data, and these results are provided in Table 15. The analysis disclosed a significant trials effect, but also a significant interaction associated with treatment and trials. Therefore, interpretation of the trials effect is dependent upon the treatment level. Furthermore, this interaction allowed the investigation of simple effects, and the summary of results for this analysis is shown in Table 16. The analysis disclosed that the formalin group showed significantly larger intakes, than the vehicle group, on the first and sixth day following injection.

Saline Intake. The mean intakes of saline for each group are shown in Table 17. Furthermore, the mean intakes for the formalin groups are illustrated in Figure 10, and for the vehicle groups in Figure 11. A 2x4x6 ANOVA with repeated measures on the third factor was utilized to analyze the data. The summary of results is presented in Table 18. The analysis revealed a significant fluid effect. A Newman-Keuls test was performed to determine where the specific differences in fluid intake occurred. The results of the test are presented in Table 19. The Newman-Keuls revealed significantly greater intakes of

Table 14

Mean Intake of Water Expressed as % B.W.  
1,2,3,4,5, & 6 Days After Injection

	<u>Time After Injection</u>					
	1	2	3	4	5	6
Formalin	16.82	10.8	13.14	12.48	13.49	14.82
Vehicle	9.05	9.98	11.48	10.11	12.25	10.48

Table 15

Analysis of Variance: Water Intake  
Expressed as % B.W.

Source	df	MS	F
<u>Between Ss</u>	13		
Treatment	1	193.19	2.94
Error <sub>(b)</sub>	12	65.7	
<u>Within Ss</u>	70		
Trials	5	14.53	5.26*
Trials X Treatment	5	24.22	8.77*
Error <sub>(w)</sub>	60	2.76	

\* $p < .05$

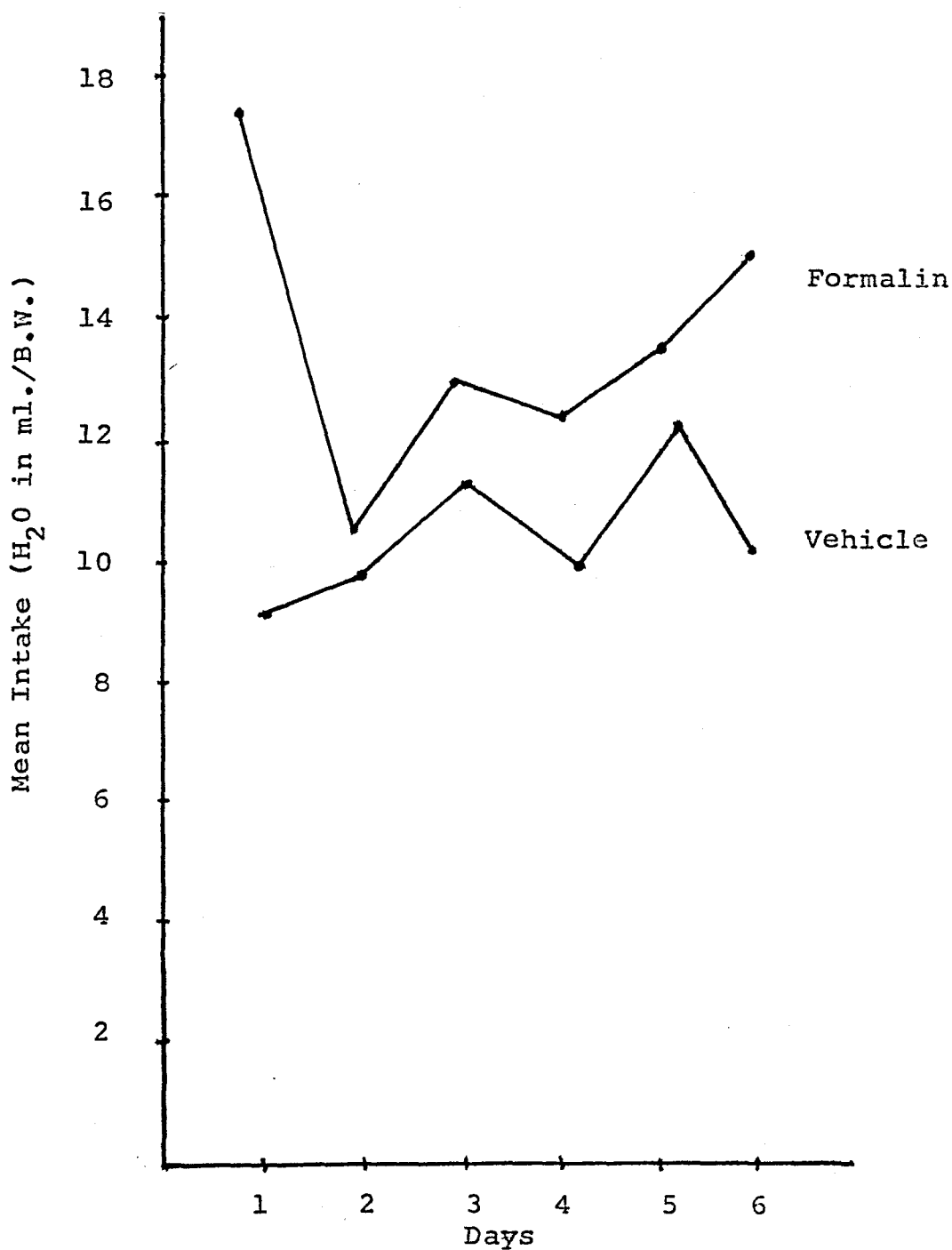


Figure 9

Mean Intakes (H<sub>2</sub>O in ml./B.W.) for the  
Formalin and Vehicle Group at 1,2,3,4,  
5, & 6 Days After Injection

Table 16Analysis of Variance: Water In-  
take Simple Effects

Source	df	MS	F
Treatments at Day 1	1	121.38	6.12*
Error	12	19.83	
Treatments at Day 2	1	11.06	.62
Error	12	17.81	
Treatments at Day 3	1	9.66	.94
Error	12	10.32	
Treatments at Day 4	1	8.29	.64
Error	12	12.98	
Treatments at Day 5	1	.44	2.28
Error	12	12.25	
Treatments at Day 6	1	65.92	4.75*
Error	12	13.87	

\*p &lt; .05

Table 17

Mean Intake of Saline Expressed as % B.W.  
1,2,3,4,5, & 6 Days After Injection

		<u>Time After Injection</u>					
		1	2	3	4	5	6
<u>Treatment; Fluid</u>	F; .45	4.3	3.25	3.31	6.0	5.0	5.54
	F; .9	3.88	1.37	2.15	.88	2.85	1.36
	F; 1.8	3.37	2.81	3.94	2.63	6.26	2.54
	F; 3.6	1.5	2.09	2.06	1.2	2.84	1.2
	V; .45	3.5	3.59	2.97	6.34	3.7	5.3
	V; .9	7.09	7.6	3.96	6.23	6.58	4.94
	V; 1.8	5.37	4.97	3.6	3.09	5.65	3.74
	V; 3.6	.7	.67	.66	.65	3.06	2.2

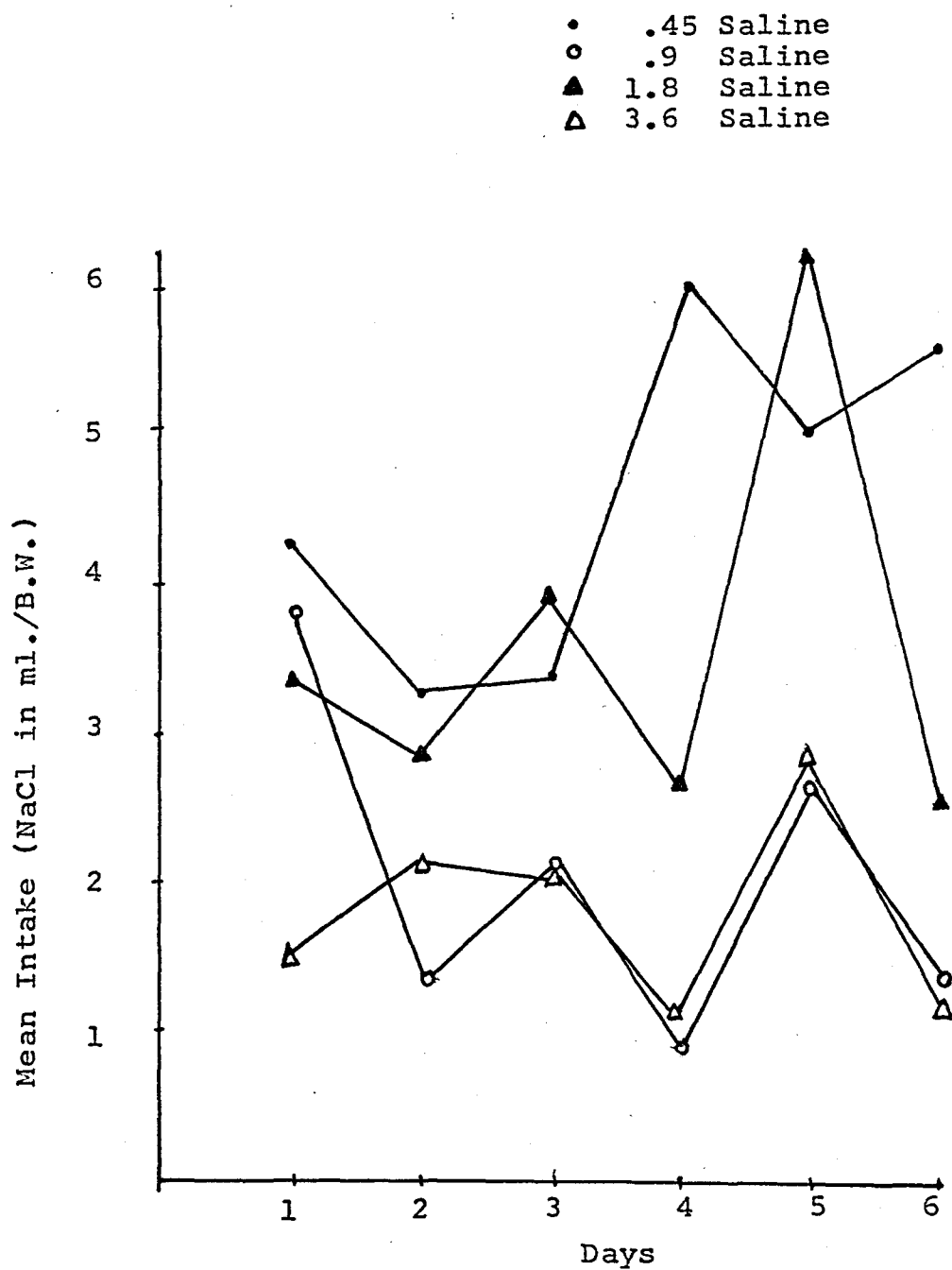


Figure 10

Mean Intakes (NaCl in ml./B.W.) for  
the Formalin Groups at 1,2,3,4,5, &  
6 Days After Injection



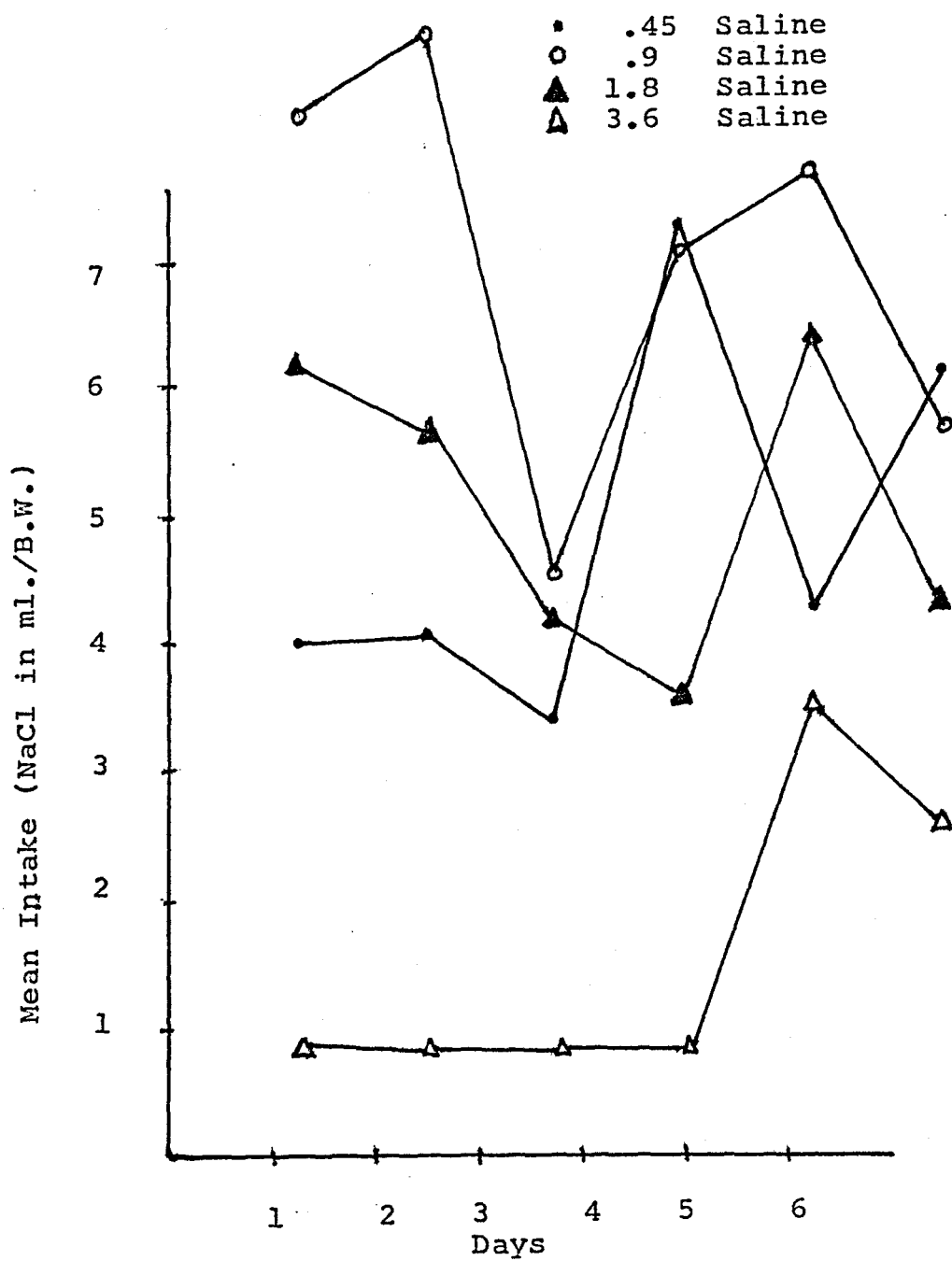


Figure 11

Mean Intake (NaCl in ml./B.W.) for the  
Vehicle Groups at 1,2,3,4,5, & 6 Days  
After Injection

Table 18

Analysis of Variance: Saline Intake Expressed  
as % B.W.

Source	df	MS	F
<u>Between Ss</u>	55		
Treatment	1	85.77	2.07
Fluid	3	144.14	3.49**
Treatment X Fluid	3	91.7	2.22
Error <sub>(b)</sub>	48	41.35	
<u>Within Ss</u>	279		
Trials	5	18.03	1.89
Trials X Treatment	5	6.71	.72
Trials X Vehicle	15	13.03	1.37
Trials X Ve- hicle X Treatment	15	4.015	.42
Error <sub>(w)</sub>	239	9.54	

Table 19

Newman-Keuls: Saline Intake  
Main Effects

<u>Trials</u>			
4	3	2	1
1.56	4.0	4.06	4.4
	2.44*	2.50*	2.84*
		.06	.4
			.34

\*\*  $p < .01$

\*  $p < .05$

.45%, .9%, and 1.8% saline, than 3.6% saline.

Water Intake. The mean intakes of water are shown in Table 20. Furthermore, the mean intakes of water for the formalin groups are depicted in Figure 12, and for the vehicle groups in Figure 13. A 2x4x6 ANOVA with repeated measures on the third factor was used to determine the statistical significance of the three factors. The summary of results is displayed in Table 21, and this analysis revealed no differences between the formalin and vehicle groups in intake of water. The mean intakes of water at 2, 4, 6, 12, and 24 hours are displayed in Table 22, and illustrated in Figure 14 for the formalin groups, and in Figure 15 for the vehicle groups. A 2x4x5 ANOVA with repeated measures on the third factor was used to analyze the data. The summary of results is presented in Table 23. The analysis revealed a significant interaction associated with treatment and trials. Due to this finding, the interpretation of the trials effect is dependent upon the treatment level. This interaction is illustrated in Figure 16. The analysis of simple effects is presented in Table 24. The analysis disclosed that the formalin groups drank significantly more water at 6 and 12 hours after injection, in comparison to the vehicle groups.

Table 20

Mean Intake of Water Expressed as % B.W.  
1,2,3,4,5, & 6 Days After Injection

		<u>Time After Injection</u>					
		1	2	3	4	5	6
<u>Treatment; Fluid</u>	V; .45	6.09	7.09	7.26	5.96	8.54	6.97
	V; .9	4.0	6.3	7.4	6.3	4.7	6.13
	V; 1.8	8.24	8.63	8.94	11.53	10.04	9.65
	V; 3.6	6.07	6.34	7.86	8.58	10.65	10.55
	F; .45	8.96	5.48	8.03	5.47	6.4	7.15
	F; .9	7.98	5.23	6.9	6.57	8.94	9.32
	F; 1.8	8.66	8.48	8.1	8.2	9.88	11.07
	F; 3.6	11.91	10.4	8.84	8.9	9.75	12.7

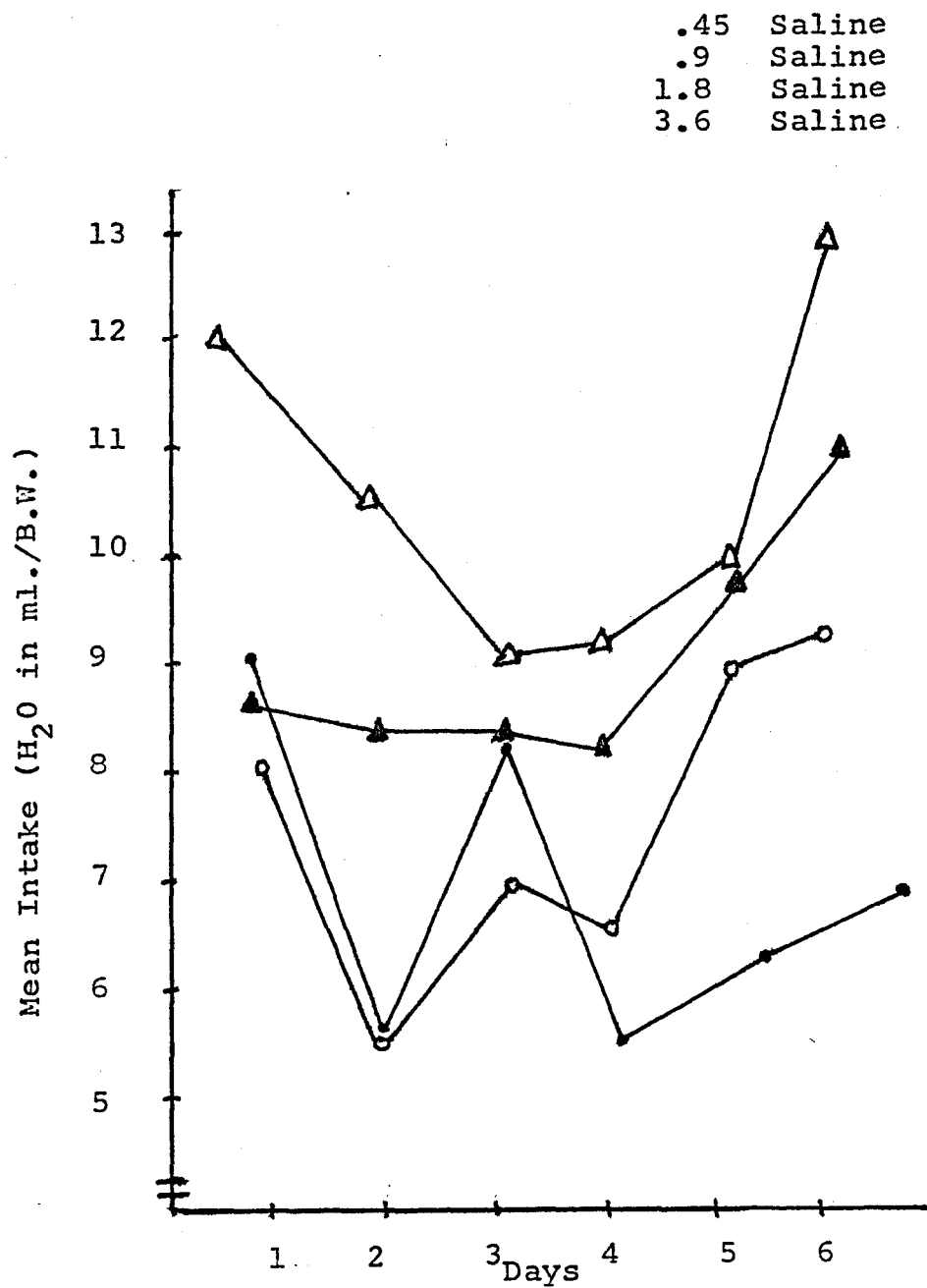


Figure 12

Mean Intake ( $H_2O$  in ml./B.W.) for the  
Formalin Groups at 1, 2, 3, 4, 5, & 6 Days  
After Injection

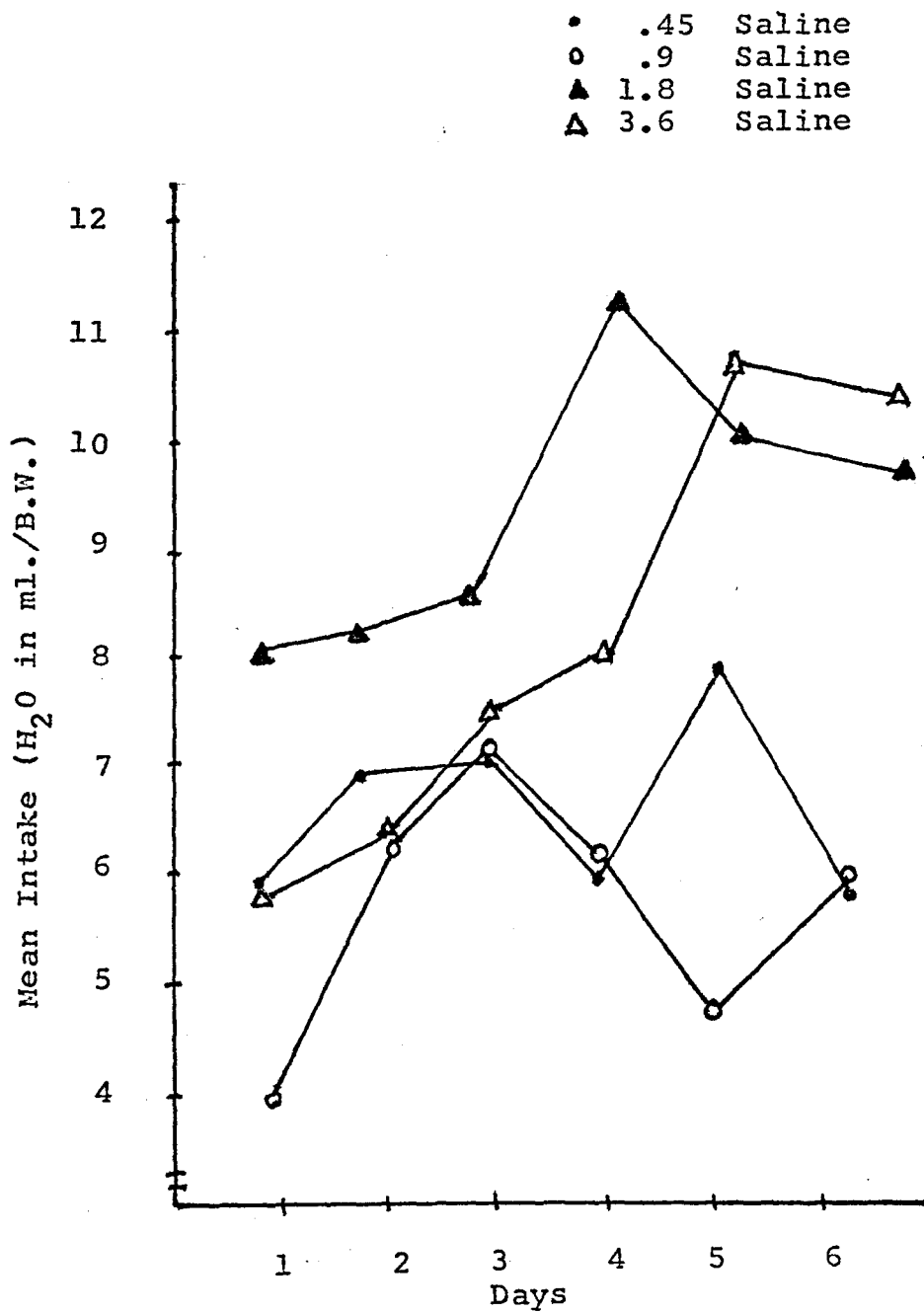


Figure 13

Mean Intake ( $H_2O$  in ml./B.W. for the  
Vehicle Groups at 1,2,3,4,5, & 6 Days  
After Injection

Table 21

Analysis of Variance: Water Intake Expressed  
as % B.W.

Source	df	MS	F
<u>Between Ss</u>	55		
Treatment	1	61.765	.613
Fluid	3	188.426	1.87
Treatment X Fluid	3	35.085	.348
Error <sub>(b)</sub>	48	100.697	
<u>Within Ss</u>	280		
Trials	5	29.48	2.17
Trials X Treatment	5	27.57	2.03
Trials X Vehicle	15	7.35	.54
Trials X Ve- hicle X Treatment	15	11.02	.81
Error <sub>(w)</sub>	240	13.61	

Table 22

Mean Intake of Water Expressed as % B.W.  
2,4,6,12, & 24 Hours After Injection

		<u>Time After Injection</u>				
		2	4	6	12	24
<u>Fluid</u> <u>Treatment:</u>	F; .45	.43	.86	1.07	2.35	4.15
	F; .9	.86	.7	.86	2.72	2.98
	F; 1.8	.43	1.02	.91	3.09	.84
	F; 3.6	.19	1.64	.81	3.38	.89
	V; .45	.21	.86	.62	1.46	2.93
	V; .9	.21	.0	.38	1.04	2.37
	V; 1.8	.2	1.49	.43	1.99	4.14
	V; 3.6	.0	.69	.0	2.25	3.13



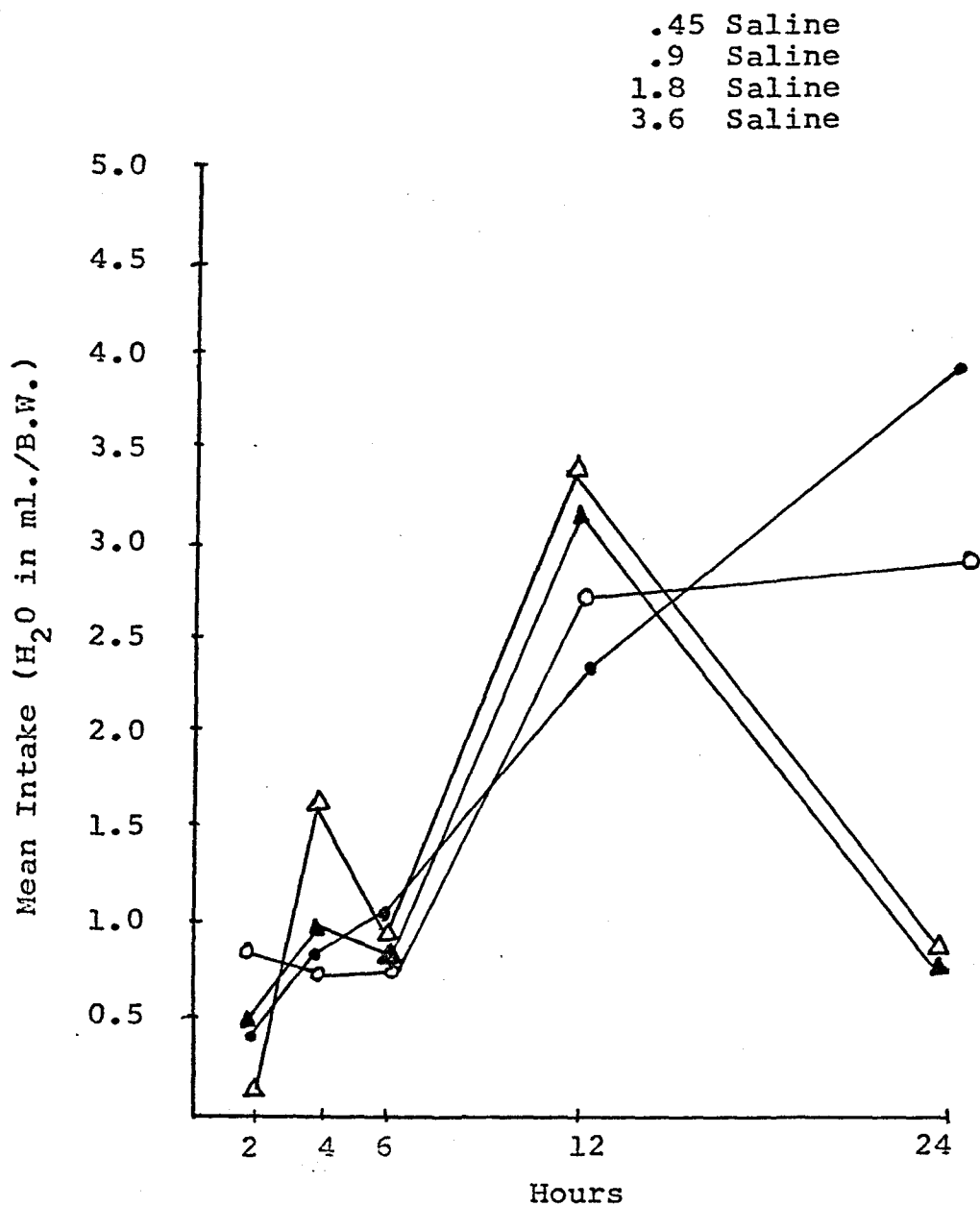


Figure 14

Mean Intakes ( $H_2O$  in ml./B.W.) for  
the Formalin Groups at 2, 4, 6, 12, &  
24 Hours After Injection

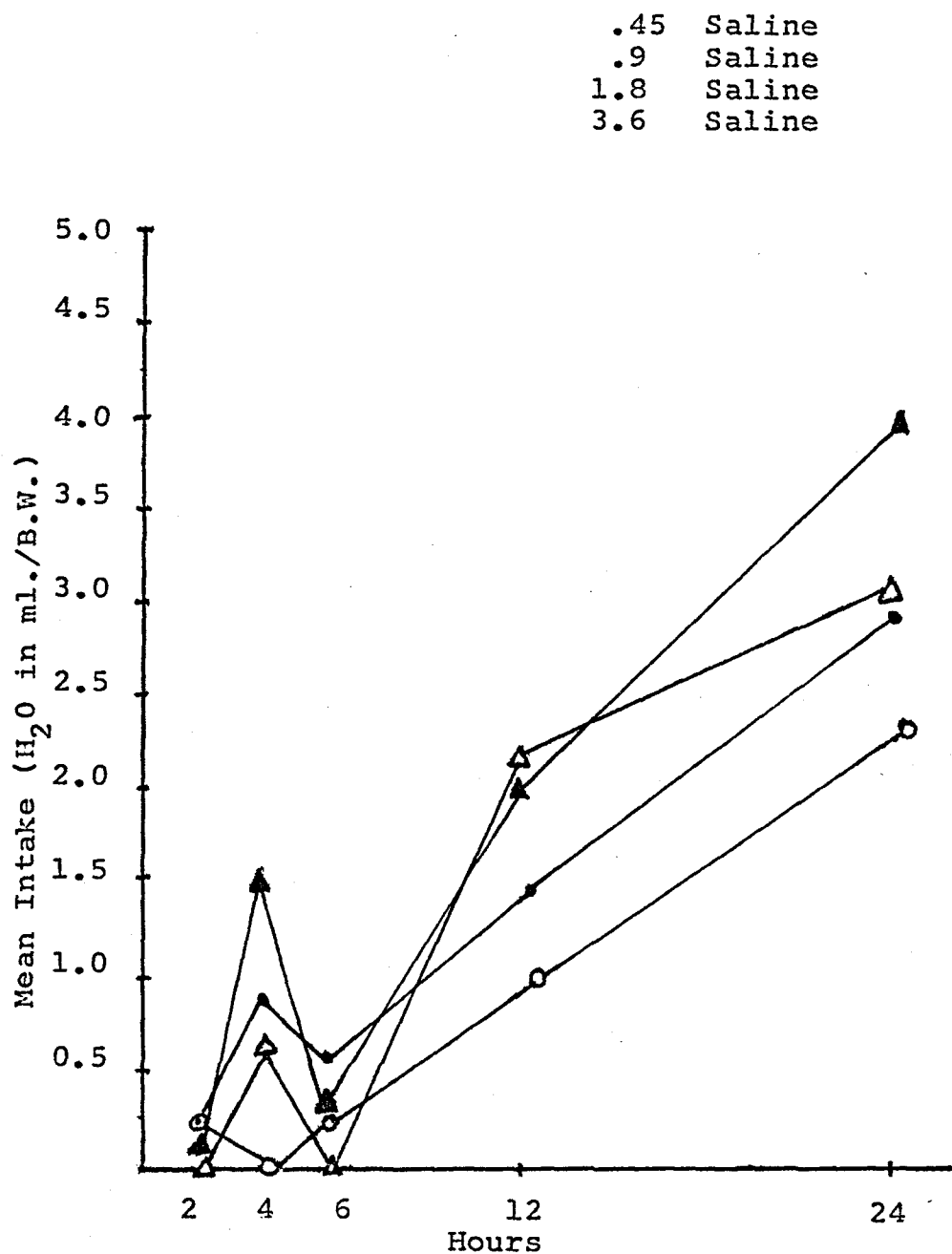


Figure 15

Mean Intake ( $H_2O$  in ml./B.W.) for  
the Vehicle Groups at 2,4,6,12, &  
Hours After Injection

Table 23

Analysis of Variance: Water Intake Expressed  
as % B.W.

Source	df	MS	F
<u>Between Ss</u>	55		
Treatment	1	5.83	1.27
Fluid	3	1.22	.27
Treatment X Fluid	3	8.05	1.75
Error <sub>(b)</sub>	48	4.59	
<u>Within Ss</u>			
Trials	4	61.79	38.66**
Trials X Treatment	4	8.33	5.21**
Trials X Vehicle	12	2.7	1.69
Trials X Vehicle X Treatment	12	2.7	1.69
Error <sub>(w)</sub>	192	1.6	

\*\*p < .01

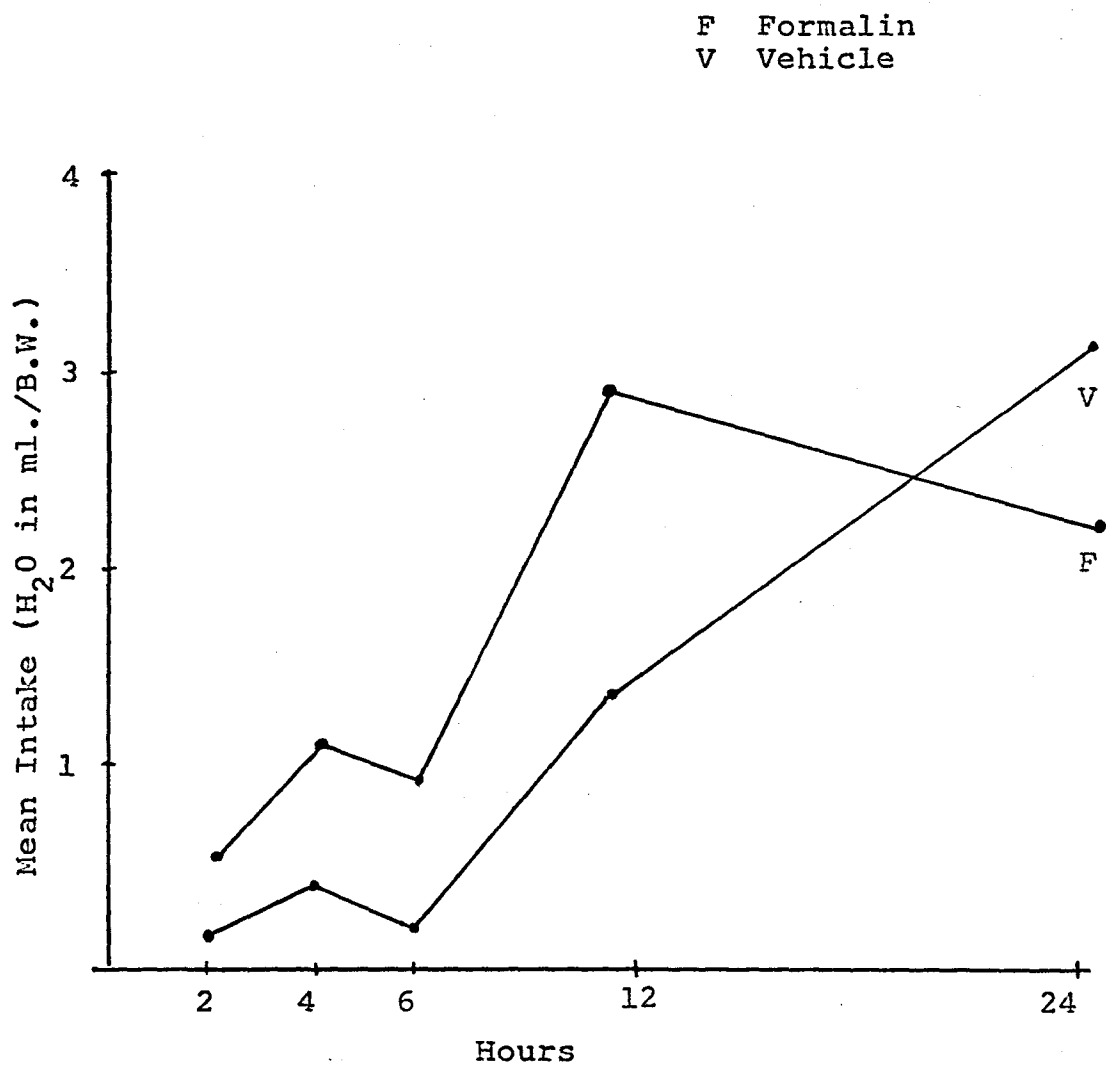


Figure 16

Mean Intake ( $H_2O$  in ml./B.W.) for Each  
Treatment<sup>2</sup> Group as a Function  
of Trials(Hours)

Table 24Analysis of Variance: Water In-  
take Simple Effects

Source	df	MS	F
Treatments at 2 Hours	1	1.46	3.24
Error	54	.45	
Treatments at 4 Hours	1	1.21	.61
Error	54	2.0	
Treatments at 6 Hours	1	6.38	10.46**
Error	54	.61	
Treatments at 12 Hours	1	20.11	8.08**
Error	54	2.49	
Treatments at 24 Hours	1	12.06	2.05
Error	54	5.89	

\*\*p &lt; .01

### Discussion

As hypothesized, this study demonstrates that gerbils significantly increase their intake of water 12 and 24 hours after formalin injection, when water is the only drinking fluid available. This suggests that the gerbil's behavioral response to acute sodium deficiency, when only permitted access to water, resembles the rat's (Stricker, 1966). Along these same lines, it was found that gerbils significantly increase their intake of water 6 and 12 hours after formalin injection, when faced with a two-bottle choice situation. However, contrary to the hypothesis, this study did not demonstrate that gerbils increase consumption of saline during acute sodium deficiency, in comparison to a vehicle group. Both treatment and vehicle groups displayed significantly larger intakes of .45%, .9%, and 1.8% saline solutions, than 3.6% saline over the six days. This difference may be attributed to the palatability of the solutions. Only the extremely hypertonic solution (3.6%), which was found to be highly unpalatable in rats, was injected less frequently. Data previously obtained in our laboratory, showed that gerbils exhibit similar preferences in saline intake (Kozub, et al, in press). Furthermore, they found that the animals never showed a preference for any of the saline solutions over water. These

findings are contradictory to Cullen (1972), who demonstrated that gerbils given either formalin or isotonic saline vehicle injections increased their intake of isotonic saline and water three to four days after injection. Here, water intake equalled that of saline or was much greater. These differences in the results of the present study and Cullen (1972) may be due to the methodological changes implemented in this study. Further evidence for this disparity arises from the findings of experiment 1. Here Cullen's results were not replicated with formalin dissolved in isotonic saline, but only with formalin dissolved in water.

The drinking responses demonstrated by the gerbil, in this study, does not replicate those seen in the rat (Stricker & Wolf, 1966; Wolf & Steinbaum, 1965; Jalowiec & Stricker, 1970). This suggests that the gerbil's behavioral and physiological response to sodium deficiency may differ. The significant increases seen only in water intake, suggest that the gerbil's drinking behavior subserves volume regulation over osmoregulation. These increases are most pronounced up to 24 hours after injection. This conclusion is dependent upon the fact that formalin injections manipulate both plasma volume and concentration, as is the case in the rat (Wolf & Steinbaum, 1965).

These physiological changes accompanying formalin injection need verification in the gerbil.

One problem leading to difficulty in interpretation of these results is the inability to take renal defense mechanisms into account, since body fluid homeostasis can best be explained in terms of the interaction between behavioral and physiological responses. As was found by Jaloweic and Stricker (1970), electrolyte need was more adequately accessed if sodium balance (intake minus excretion) were measured, rather than sodium appetite. In regard to this study, the differences between treatment and vehicle groups may have been in terms of renal output. The vehicle groups may not have shown any evidence of sodium retention. Here, the intake of saline displayed by this group would not produce any effect on body fluid homeostasis, since renal mechanisms could dispose of the excess sodium. On the other hand, formalin treated animals, who also ingested similar amounts of sodium, could remediate their deficits by decreasing urine volume and concentration. As a result, only this group may have actively retained the sodium, and thereby replenished their deficits.

At present, only one model delineating the mechanisms of sodium appetite exists. According to the reservoir hypothesis of Wolf & Stricker (1967), sodium



appetite is elicited only when the sodium content of the reservoir has been depleted. Therefore, sodium appetite would be less responsive to changes in intravascular fluid volume and concentration. If the gerbil's behavioral response is to replenish their volume deficit first, then it is possible that the concentration deficit is remediated via the reservoir. The small amounts of saline injected on a daily basis may serve to replenish the reservoir, and at the same time does not increase the osmolarity of the body fluids. Furthermore, since no great increase in saline consumption was evident, it is possible the reservoir's sodium was not totally diminished, suggesting that the animal may be more resistant to electrolyte imbalance.

Another explanation is that the gerbil is unable to regulate sodium intake. Therefore, the increases in water intake may have actually been due to hyponatremia. Also, it may be possible that formalin does not elicit a sodium appetite in the gerbil, but only produces a nonspecific thirst. Therefore, the increases in water intake may be to remediate this thirst.

In summary, the gerbil's behavioral response to acute sodium deficiency is to increase water consumption to remediate the plasma volume deficit. However, due to the similar behavior of the formalin and the

vehicle groups, no definitive conclusion can be made concerning the gerbil's ability to regulate sodium intake, when various concentrations of saline solutions and water are provided. It may be the case that the gerbil can only regulate when saline solutions of various concentrations are provided without the presence of water.

### Summary

The results of experiment 1 demonstrated that the effects of formalin is dependent upon the type of vehicle in which formalin is dissolved. In particular, gerbils respond to formalin dissolved in water by increasing saline intake and decreasing water intake in comparison to the vehicle injection. On the other hand, formalin dissolved in saline did not produce a similar effect. Here, no differences in saline intake were observed, but the formalin group drank significantly more water in comparison to its vehicle group.

Experiment 2, demonstrated that the gerbils significantly increase water consumption 12 and 24 hours after formalin injection, when water is the only drinking fluid available. Furthermore, gerbils significantly increase their intake of water 6 and 12 hours after formalin injection when various concentrations of saline solutions and water are provided. At the same time, no increases in saline consumption

were observed, but both treatment and vehicle groups drank larger amounts of .45%, .9%, and 1.8% saline, than 3.6% saline. The results of this experiment suggests that either formalin does not elicit a sodium appetite in the gerbil, or that the gerbil regulates sodium and water in a manner different from the rat during acute sodium deficiency.

At present, it is difficult to account for the contradictory findings of experiment 1 and 2. Replication is needed to verify the validity of the behavioral responses of the gerbil found in this study. Only future research can delineate the specific mechanisms and the physiological changes involved in water and sodium appetite. The speculations made in this regard need to be substantiated. Future research should also examine the role of physiological mechanisms and its relationship to the observed behavioral responses, since both are involved in the regulation of body fluid homeostasis. Various blood and urine analyses need to be performed to verify the physiological effects of formalin in the gerbil, and to substantiate the remedial effects of sodium and water intake. Lastly, further research should be conducted to examine the gerbil's response to sodium appetite produced by other experimental manipulations of electrolyte balance and to increases in mineralocorticoid levels.

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